The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Test Plan for Monoterpene Hydrocarbons

d-Limonene	CAS No. 5989-27-5
dl-Limonene	CAS No. 138-86-3
Terpinolene	CAS No. 586-62-9
Myrcene	CAS No. 123-35-3
Dihydromyrcene	CAS No. 2436-90-0
Hydrocarbons, terpene processing by-products	CAS No. 68956-56-9
Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	CAS No. 8008-57-9
Terpenes & terpenoids, sweet orange oil	CAS No. 68647-72-3
Terpenes & terpenoids, turpentine oil, limonene fraction	CAS No. 65996-99-8
Terpenes & terpenoids, limonene fraction	CAS No. 65996-98-7
Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	CAS No. 68334-40-7
Terpenes & terpenoids, turpentine-oil residue	CAS No. 68938-00-1

FFHPVC Terpene Consortium Registration Number

List of Member Companies

ARIZONA CHEMICAL

BASF CORPORATION

BEDOUKIAN RESEARCH, INC.

BOISE CASCADE CORPORATION

CHAMPION INTERNATIONAL CORPORATION

CITRUS & ALLIED ESSENCES, LTD.

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FRAGRANCE RESOURCES, INC.

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POLAROME INTERNATIONAL INCORPORATED

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SENSIENT FLAVORS

TECNAL CORPORATION

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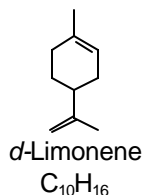# The HPV Challenge Test Plan for Monoterpene Hydrocarbons

1 Identity of Substances

d-Limonene
$C_{10}H_{16}$

Synonyms:
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-
(R)-(+)-p-Mentha-1,8-diene
d-1-Methyl-4-isopropenyl-1-cyclohexene

CAS No. 5989-27-5

dl-Limonene
$C_{10}H_{16}$
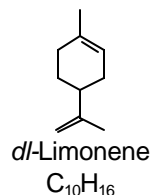
Synonyms:
Cyclohexene, 1-methyl-4-(1-methylethenyl)-
Dipentene

CAS No. 138-86-3

Terpinolene
$C_{10}H_{16}$
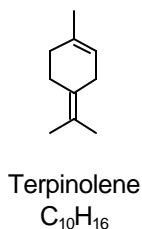
Synonyms:
Cyclohexene, 1-methyl-4-(1-methylethylidene)-
p-Mentha-1,4(8)-diene
1-Methyl-4-isopropylidene-1-cyclohexene

CAS No. 586-62-9
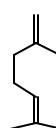
Myrcene
$C_{10}H_{16}$

Synonyms:
1,6-Octadiene, 7-methyl-3-methylene-
7-Methyl-3-methylene-1,6-octadiene
beta-Myrcene

CAS No. 123-35-3

Dihydromyrcene
$C_{10}H_{18}$
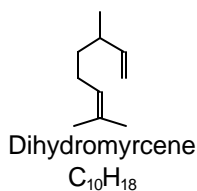
Synonyms:
1,6-Octadiene, 3,7-dimethyl-
3,7-Dimethylocta-1,6-diene

CAS No. 2436-90-0

22-34%   22-33%   5-10%   18%   10%
limonene terpinolene myrcene
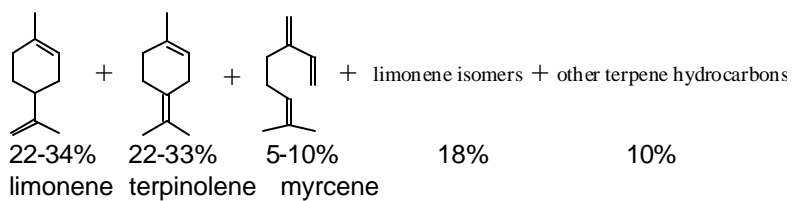
Hydrocarbons, terpene processing by-products

CAS No. 68956-56-9
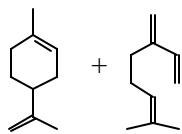
91-94 %   2.0-2.1%
Limonene   *beta*-Myrcene

Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)

CAS No. 8008-57-9
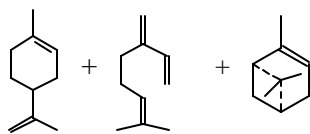
d-Limonene *beta*-Myrcene   *alpha*--Pinene
91-95%   1-3%   1-2%

Terpenes & terpenoids, sweet orange oil
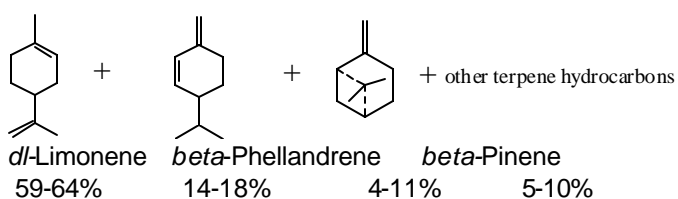
CAS No. 68647-72-3

dl-Limonene   *beta*-Phellandrene   *beta*-Pinene

59-64%   14-18%   4-11%   5-10%

Terpenes and terpenoids, turpentine oil, limonene fraction

CAS No. 65996-99-8

96-98%   1-2%   5-10%

dl-Limonene   Myrcene

Terpenes and terpenoids, limonene fraction

CAS No. 65996-98-7

dl-Limonene   terpinolene

30-35%   1-3%   30-35%   15-20%   5-12%

Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue

CAS No. 68334-40-7

3

d-Limonene   beta-Phellandrene   beta-Myrcene

+ pinene, camphene and delta-3-carene + nonvolatiles and polymers

40%

60%

8% volatiles

92%
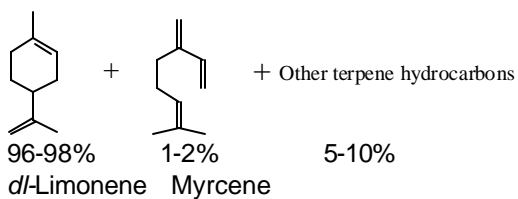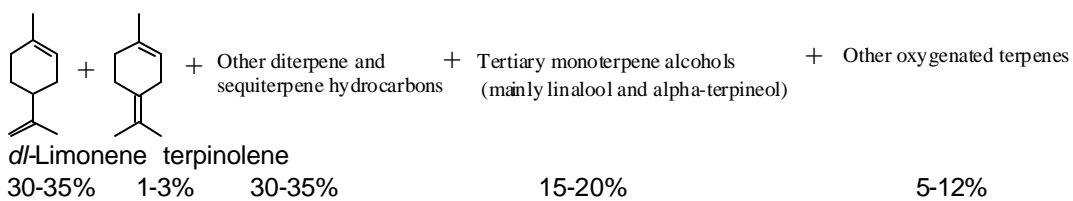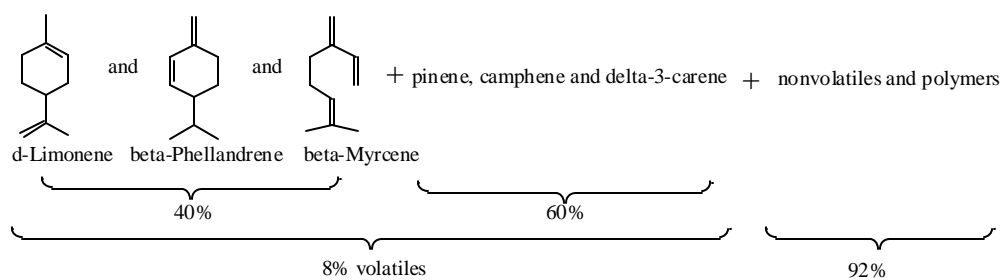
Terpenes & terpenoids, turpentine-oil residue

CAS No. 68938-00-1

2 Category Analysis

2.1 Introduction

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Terpene Consortium, as a member of FFHPVC serves as an industry consortium to coordinate testing activities for terpenoid substances under the Chemical Right-to-Know Program. Twenty-one (21) companies are current members of The Terpene Consortium. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The category analysis, test plan, and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 Background Information

The chemical category designated "Monoterpene Hydrocarbons" includes five simple monoterpene hydrocarbons and seven mixtures comprised primarily of the five terpene hydrocarbons. In plants, monoterpene hydrocarbons are produced by the isoprene pathway. Monoterpene hydrocarbons have a chemical formula of $C_{10}H_{16}$, or if partly or completely saturated, $C_{10}H_{18}$ or $C_{10}H_{20}$. Monoterpene hydrocarbons are ubiquitous in food [CIVO-TNO, 1999] given that they are present in varying degrees in all plants. Being volatile constituents of plants, they are also normal components of the atmosphere.

Monoterpene hydrocarbons are mainly released by coniferous woodland such as pine trees, cedars, redwood and firs. To a lesser extent, they are also produced and released by deciduous

plants. They are common components of traditional foods occurring in essentially all fruits and vegetables [CIVO-TNO, 1999]. *d*-Limonene, *beta*-myrcene, and terpinolene are currently recognized by the U.S. Food and Drug Administration (FDA) as GRAS ("generally regarded as safe") for their intended use as flavoring substances [Hall and Oser, 1965]. Quantitative natural occurrence data indicate that oral intake of these substances occurs predominantly from consumption of food in which they occur naturally [Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985]. Greater than 2,500,000 pounds (lbs) of *d*-limonene, 50,000 lbs of terpinolene, and 150,000 lbs of *beta*-myrcene are consumed annually as natural components of food in the United States. The estimated poundage of *d*-limonene, terpinolene and *beta*-myrcene used as flavoring substances in 1995 were 213,000 lbs, 1,170 lbs, 2,620 lbs, respectively [Lucas *et al.*, 1999]. Therefore, greater than 90%, 97% and 98% of intake occurs from consumption of food containing naturally occurring *d*-limonene, terpinolene and *beta*-myrcene, respectively. Based on the annual volume of consumption of *d*-limonene, terpinolene and *beta*-myrcene, it is estimated that the combined average daily *per capita* intake is approximately 1.3 mg/day. Intakes as high as 13 mg/day (eaters only) may be expected for consumers of diets rich in fruits, vegetables, and spices [Oser and Hall, 1977].

As a volatile C_{10} hydrocarbon, limonene is also a naturally occurring component of the atmosphere. Estimates of atmospheric concentrations of limonene in urban indoor air, rural outdoor air (*Pinus* forest canopy), and occupational environments (*e.g.* sawmill or paper mill worker) have been reported to be approximately 0.6-11.1 microgm/m^3, 0.6-1.1 microgm/m^3, and 1.7-240 microgm/m^3, respectively [IPCS, 1998]. Assuming that a human is exposed daily to an urban atmosphere containing 11 ug/m^3 *d*-limonene and that 65% of the inhaled *d*-limonene is absorbed [Falk-Filipsson *et al.*, 1993], the daily intake from atmospheric exposure would be approximately 0.5 mg/day {11 ug/m^3 x 3 m^3/hr x 24hrs/day x 0.65 (absorption rate) x 10^{-3} ug/mg}. When oral and inhalation exposures are combined, it is estimated that average total daily exposure from food consumption and normal inhalation in an urban environment is in the range of 2 mg. However, for specialized eating groups (90% eaters, *e.g.,* vegetarians), daily intakes may easily exceed 20 mg.

2.3 Structural Classification

The chemical category designated terpenoid hydrocarbons includes three simple C_{10} isomeric monocyclic terpene hydrocarbons (*d*-limonene, *dl*-limonene, and terpinolene) two simple C_{10} acyclic terpene hydrocarbons (*beta*-myrcene and dihydromyrcene) and seven mixtures composed primarily of *d*-limonene, *dl*-limonene (dipentene), terpinolene, myrcene, and *alpha*- and *beta*-pinene (see the FFHPVC Test Plan for the Chemical Category Bicyclic Terpenoid Hydrocarbons). *d*-Limonene and terpinolene are monocyclic monounsaturated terpenes. *d*-Limonene is (R)-1-methyl-4-(1-methylethenyl)-cyclohexene, *dl*-limonene is an equal mixture of (R)- and (S)-1-methyl-4-(1-methylethenyl)-cyclohexene while terpinolene is 1-methyl-4-(1-methylethylidene)-cyclohexene. Myrcene is commonly recognized as *beta*-myrcene, the isomeric form that predominates in nature. *beta*-Myrcene is an acyclic monounsaturated isomer of limonene. The *alpha* isomer, 2-methyl-6-methylene-1,7-octadiene is not found in nature [Merck, 1996] and is of no commercial importance. *beta*-Myrcene is 7-methyl-3-methylene-1,6-octadiene while dihydromyrcene is 3,7-dimethyl-1,6-octadiene.

Typical analyses of the seven mixtures in this category reveal that the five chemically defined members of this chemical category are major constituents. Hydrocarbons, terpene processing by-products is primarily composed of limonene, isomers of limonene, terpinolene, myrcene and other terpene hydrocarbons [Arizona Chemical, 1999]. Orange peel oil, sweet (Citrus sinensis (L.) Osbeck) is composed almost completely of *d*-limonene (91-94%) with *beta*-myrcene as a minor constituent (2.0-2.1%). Terpenes and terpenoids, sweet orange oil is primarily *d*-limonene (91-95%), *beta*-myrcene (1-3%), and *alpha*-pinene (1-2%) [Bauer K. and D. Garbe, 1985]. Terpenes and terpenoids, turpentine oil, limonene fraction is primarily racemic (*dl*)-limonene (59-64%), with *beta*-phellandrene (1-methyl-4-isopropyl-1,5-cyclohexadiene,14-18%), *beta*-pinene (4-11%) and other terpene hydrocarbons (5-10%) being minor constituents [Arizona Chemical, 1999]. Terpenes and terpenoids, limonene fraction is composed primarily of racemic limonene (96-98%), myrcene (1-2%) and other terpene hydrocarbons (5-10%) [Arizona Chemical, 1999]. Terpenes and terpenoids, turpentine oil, limonene fraction, distillation residue is composed of limonene (30-35%), other diterpene and

sesquiterpene hydrocarbons (30-35%), tertiary monoterpene alcohols (15-20%), terpinolene (1-3%) and other oxygenated terpenes (5-12%) [Arizona Chemical, 1999]. Terpenes and terpenoids, turpentine-oil residue is composed of polymeric (82%), and nonvolatile terpene (10%) constituents which have no commercial value. This residue is used as fuel at the industrial site at which it is isolated from turpentine. The volatile component (8%) is composed of approximately 40% limonene, phellandrene, and myrcene isomers and 60% pinene, camphene and carene isomers [Arizona Chemical, 2000].

2.4 Industrial and Biogenic Production

2.4.1 Industrial Production

d-Limonene is a liquid with a lemon-like odor and a byproduct from the manufacture of orange juice. It may also be obtained from orange oils through vacuum distillation. It has been estimated that the worldwide production of orange oil (*d*-limonene accounts for greater than 90% of orange oil) is 26,000 tons, with the greatest production occurring in Brazil (17,000 tons) and in the United States (6,900 tons) [Lawrence, 1985]. Limonene is also produced by acid catalyzed isomerization of *alpha-* and *beta*-pinene [Bauer and Garbe, 1985]. Myrcene is largely produced from the pyrolysis of *beta*-pinene at high temperatures [Bauer and Garbe, 1985].

Another industrial source of limonene and *beta*-myrcene is crude sulfate turpentine (CST) obtained as a waste product in the manufacturer of cellulose *via* the sulfate process. Turpentine is derived primarily from *Pinus* species and is used in whole form as a solvent for paints and varnishes or as a cleaning agent. Turpentine is composed of approximately 60-65% *alpha*-pinene, 25-35% *beta*-pinene with the remainder being other terpenoid hydrocarbons including limonene and myrcene. CST obtained from southern paper mills in the United States consists of 4.2% *d*-limonene and 1.7% *beta*-myrcene [Derfer and Traynor, 1992]. It has been estimated that the worldwide production of turpentine is approximately 330,000 metric tons of which almost 100,000 metric tons is gum turpentine and the bulk of the remainder is sulphate turpentine [National Resources Institute, 1995].

Level 1 fugacity calculations using limonene indicate that 93.8% will partition to air at equilibrium. In the atmosphere, limonene rapidly reacts with hydroxyl radicals, ozone and nitrate radicals [NICNAS, 2001]. If it were conservatively assumed that 2% of industrially separated limonene is lost during industrial processing of orange oil and turpentine, the total annual worldwide emission of *d*-limonene from these industrial sources would be approximately 800 metric tons. This can be compared with the biogenic emissions into the air discussed below.

Limonene derived from citrus essential oils and CST, is used as a raw material for the chemical synthesis of a variety of terpene alcohols and ketones such as menthol, carvone and *alpha*-terpineol [Bauer and Garbe, 1985]. Limonene is also used directly but in far less amounts as a fragrance material for household products and as a component in the manufacture of artificial essential oils [Lawrence, 1985].

2.4.2 Biogenic Production

d-Limonene, terpinolene and myrcene naturally occur in many essential oils. Very high levels of *d*-limonene are present in orange oil (greater than 90%), grapefruit oil (90%), lemon oil (70%) and celery oil (60%) etc. [NICNAS, 2001]. *beta*-Myrcene and terpinolene occur naturally in a wide variety of foods including lemon peel oil, orange peel oil, orange juice, and lime juice [CIVO-TNO, 1999].

In a recent study of the measurement of terpene emissions from *Pinus sylvestris* dominated forests [Rinne *et al.*, 2000], it was reported that the main monoterpenes emitted were *alpha*-pinene (57%), *delta*-3-carene (22%), *beta*-pinene/myrcene (14%), limonene (5%) and camphene (3%). The emissions of *d*-limonene and myrcene are not limited to conifers. In a study of emissions over arable crops and a beech forest [Gallagher *et al.*, 2000], all five substances were detected. Indeed landscape flux potentials have been measured in three quite varied sites (an urban forest, a mixed deciduous and coniferous forest, and a mixed shrub oak forest) in the U.S. from each of 63 species of trees [Helmig *et al.*, 1999a, 1999b]. *d*-Limonene, *beta*-myrcene and terpinolene were detected in a substantial proportion of the species measured with fluxes ranging from 0.1 to 67 μgChr^{-1}gdw^{-1} (μg carbon per hour per gram dry

weight), 0.1 to 2.6 µgChr^{-1}gdw^{-1} and 0.1 to 2.2 µgChr^{-1} gdw^{-1}, respectively [Helmig *et al.,* 1999a]. These fluxes have been used to calculate average daily fluxes for each substance at each site [Helmig *et al.*, 1999b]. For *d*-limonene these were 71, 120 and 15 µgCm^{-2}hr^{-1} (µg carbon per m^2 per hour), for *beta*-myrcene, 14, 14 and 0.4 µgCm^{-2}hr^{-1} and for terpinolene, 5, 15 and 2 µgCm^{-2}hr^{-1}, as measured above an urban forest, a mixed deciduous and coniferous forest, and a mixed shrub oak forest, respectively. The relative emissions for *d*-limonene is 3.6, 2.7 and 0.6%; for *beta*-myrcene, 0.7, 0.3 and 0.01%; and for terpinolene, 0.3, 0.3 and 0.1% of the total volatile organic compounds (VOC) emissions for each of the three sites, respectively. These figures can be used to estimate the total global emissions of these materials (see below).

In a recent review of natural emissions of volatile compounds [Guenther *et al.*, 2000] it was estimated that in North America the total annual emission of *d*-limonene and myrcene[1] was 0.4 to 1.1 million metric tons; the total annual emission of terpinolene[2] was 0.1 to 0.4 million metric tons. The total global emissions of these three compounds can be estimated in two ways. The total annual global emission of VOCs has been estimated as 1,150 million metric tons [Guenther *et al.*, 1995]. If the same percentage of total emissions of VOCs as has been measured over 3 different forest types, 3.6, 2.7 and 0.6% for *d*-limonene (average = 2.3%), 0.7, 0.3 and 0.01% (0.34%) for *beta*-myrcene and 0.3, 0.3 and 0.1% (0.23%) for terpinolene, it can be estimated that the total annual global emissions for these three substances would be approximately 26.5 million, 4 million and 2.6 million metric tons, respectively. On the other hand, if the average rates of emission of *d*-limonene (69 µgCm^{-2}hr^{-1}), *beta*-myrcene (9.5 µgCm^{-2}hr^{-1}) and terpinolene (7.3 µgCm^{-2}hr^{-1}) are applied to the latest global forest coverage estimates of 3.9 billion hectares [Food and Agriculture Organization, 2000], then annual global biogenic emissions of *d*-limonene, *beta*-myrcene and terpinolene are approximately 23.5 million, 3.2 million and 2.5 million metric tons, respectively.

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[1] Emission rate was estimated for d-limonene, sabinene, β-phellandrene, p-cymene and myrcene [Guenther *et al.*, 2000].

[2] Emission rate was estimated for camphene, camphor, bornyl acetate, α-thugene, terpinolene, α-terpinene, γ-terpinene, ocimene, 1,8-cineole, piperitone, α-phellandrene, and tricyclene [Guenther *et al.*, 2000].

Based on the close agreement of estimates of biogenic emissions derived from two separate methods, it is concluded that total annual atmospheric emission of *d*-limonene, *beta*-myrcene and terpinolene is predominantly from biogenic sources. The relative contribution from biogenic and industrial sources can be represented by a global emission ratio (GER = biogenic emission/industrial emission). In the case of *d*-limonene, the GER would exceed 10,000, suggesting that biogenic emissions far exceed anthropogenic emissions. As a result, humans are unavoidably exposed to naturally occurring monterpenoid hydrocarbons including limonene, myrcene and terpinolene.

2.5 Chemical Reactivity and Metabolism

Studies of terpene hydrocarbons indicate that they are rapidly absorbed, distributed, metabolized and excreted. The principal metabolic pathway involves side chain oxidation to yield monocyclic terpene alcohols and carboxylic acids. These metabolites are mainly conjugated with glucuronic acid and excreted in the urine, or to a lesser extent in the feces. A secondary pathway involves epoxidation of either the exocyclic or endocyclic double bond yielding an epoxide that is subsequently detoxicated *via* formation of the corresponding diol or conjugation with glutathione. Although some species- and sex-specific differences exist, studies for *d*-limonene and *beta*-myrcene indicate that the monoterpene hydrocarbons in this chemical category will participate in common pathways of absorption, distribution, metabolism and excretion.

2.5.1   Absorption, Distribution and Excretion

Following oral intake, limonene is completely and rapidly absorbed and distributed throughout the body, preferentially to fatty tissues, as shown by a high oil blood partition coefficient and a long half life during the slow elimination phase [Falk *et al.*, 1990; Falk-Filipsson *et al.*, 1993]. Volunteers exposed *via* inhalation to 450 mg/m^3 *d*-limonene showed three phases of elimination in the blood, with half-lives of about 3, 33, and 750 minutes, respectively [Falk-Filipsson *et al.*,

1993]. Radioactivity in the liver, kidney and blood were negligible 48 hours after oral administration [^{14}C]-*d*-limonene to rats. About 60% of the administered radioactivity was recovered from the urine, with 5% from feces and 2% from expired CO_2 [Igimi *et al.*, 1974]. In a separate study using male human volunteers, 50-80% of an oral dose of ^{14}C-*d*-limonene was excreted in the urine with less than 10% appearing in the feces [Kodama *et al.*, 1976].

2.5.2   Biotransformations

Limonene is metabolized *via* cytochrome P450 to produce polar metabolites, which are conjugated and excreted in the urine. The major metabolic pathway involves oxidation of the 1-methyl group yielding perillic acid [Kodama *et al.*, 1976] (see Figure 1). Limonene is also epoxidized at the 8,9-double bond. The resulting epoxide is hydrolyzed to the corresponding diol by epoxide hydrolase (EH) [Watabe *et al.*, 1981]. Other studies have also indicated minor pathways involving ring hydroxylation and epoxidation of the 1,2-double bond [Kodama *et al.*, 1976; Poon *et al.*, 1996].

2.5.3   Humans

Limonene given orally to humans yields the following major plasma metabolites: perillic acid, limonene-1,2-diol, limonene-8,9-diol, and dihydroperillic acid, probably derived from perillic acid [Poon *et al.*, 1996; Crowell *et al.*, 1994; Vigushin *et al.*, 1998]. Limonene (unchanged) and perillic acid artifacts (methyl ester) were also detected as minor plasma metabolites [Poon *et al.*, 1996]. Peak plasma levels for all metabolites were achieved 4-6 hours after administration, with the exception of limonene-8,9-diol which reached its peak level one hour after administration [Crowell *et al.*, 1994]. Phase II glucuronic acid conjugates have been identified in the urine of human volunteers for all major and minor metabolites. They include the glucuronic acid conjugates of perillic acid, dihydroperillic acid, limonene-8,9-diol, limonene-10-ol, limonene-6-ol, and limonene-7-ol (perillyl alcohol) [Poon *et al.*, 1996; Kodama *et al.*, 1974; 1976].

2.5.4 Metabolism in Rats and Other Animals

Similar to humans, the C_1 methyl substituent of limonene is oxidized in the rat to form perillic acid (see Figure 1). Perillic acid can then be excreted in the urine unchanged or as the glycine or glucuronic acid conjugate or it can be further oxidized to perillic acid-8,9-diol or 2-hydroxy-*p*-menth-8-en-7-oic acid. Approximately 85% of the urinary limonene metabolites in the rat were identified as perillic acid or a metabolite of perillic acid [Kodama *et al.*, 1976]. Minor pathways reported for the rat include epoxidation of either the 1,2- or the 8,9- double bond, and subsequent hydrolysis to the diol.

Figure 1. Metabolism of *d*-limonene in Animals

perillic acid perillic acid-8,9-diol

limonene-8,9-epoxide limonene-8,9-diol
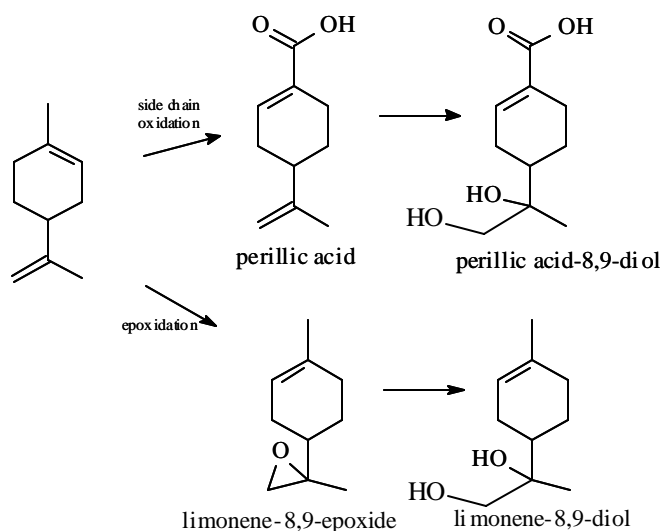
Upon incubation with rat liver microsomes, the majority of *d*-limonene was converted to the 8,9-diol *via* its precursor the 8,9-epoxide and to a lesser extent, the 1,2-epoxide. Further

evidence of conversion of the 8,9-diol *via* the 8,9-epoxide was provided when 3,3,3-trichloropropene-1,2-oxide, an inhibitor of epoxide hydrolase, completely blocked the NADPH-dependent microsomal hydrolysis of the 8,9-epoxide to the 8,9-diol as shown by the accumulation of the 8,9-epoxide in the reaction medium. Epoxidation of the C_8-C_9 double bond is favored over epoxidation of the C_1 double bond, due to the steric hindrance of the 1-methyl group. The 1,2-epoxide underwent a very low rate of microsomal hydrolysis (1% of the rate for the 8,9-epoxide) which explains the absence of the 1,2 diol as a microsomal metabolite [Watabe *et al.*, 1981].

Incubation of male rat liver microsomes with either *d*-or *l*-limonene resulted in the formation of the corresponding perillyl alcohol and carveol stereoisomers. Cytochrome P-450 2C11 catalyzes formation of the alcohol metabolites. However, incubation of limonene with female rat liver microsomes resulted in lower activity for conversion to either alcohol. Use of phenolbarbital induced liver microsomes resulted in an increase in carveol metabolites. With fetal liver microsomes, rates of limonene hydroxylation were low or undetectable. After birth, limonene hydroxylation increased in males but not in females, and the formation of perillyl alcohol increased the most rapidly. The authors concluded that sex-related differences in metabolism may provide a basis for understanding the sex-specific chronic nephrotoxicity (see 3.4.4 Repeat Dose Toxicity) reported in Sprague Dawley rats [Miyazawa *et al.*, 2002].

In male rats orally administered 3 mmol/kg (408 mg/kg) of [C^{14}]-*d*-limonene, the 1,2-epoxide (82%), the 1,2-diol (5%), and *d*-limonene (13%) were detected in the renal proximal tubular cells where they were reversibly (40%) associated with *alpha*-2-globulin, a hepatic protein filtered by the glomeruli. It has been determined that these protein-*d*-limonene metabolite associations in the P-2 section of the proximal tubule are a prerequisite for the observed nephrotoxicity in the male rat [Lehman-McKeeman *et al*., 1989]. No such *alpha*-2-globulin has been observed in man [Olson *et al*., 1990].

Urinary metabolites isolated from male rabbits orally administered [^{14}C]-*d*-limonene included perillic acid-8,9-diol (major), *p*-menth-1,8-dien-10-ol, *p*-menth-1-ene-8,9-diol, perillic acid, *p*-

mentha-1,8-dien-10-yl glucuronic acid and 8-hydroxy-*p*-menth-1-en-9-yl- *beta*-glucopyranosiduronic acid [Kodama *et al.,* 1974].

Similar to limonene, myrcene participates in the epoxidation pathways. In male rabbits given 400-700 mg/kg bw [^{14}C]- *beta*-myrcene by gavage, the principal urinary metabolites identified were myrcene-3,10-glycol (40.7%), myrcene-1,2-glycol (20.8%), and uroterpenol (11.8%), illustrating that myrcene may undergo epoxidation of the 3,10-double bond (terminal double bond), epoxidation of the 1,2-double bond, or ring closure to uroterpenol, presumably *via* limonene that was derived from myrcene in the acidic conditions of rabbit stomachs. Similarly to data for *d*-limonene, epoxidation of the 3,10-double bond was favored over epoxidation of the 1,2-double bond [Ishida *et al.*, 1981].

In Phase I metabolism, the biotransformation of *d*-limonene and myrcene as well as the other category members are catalyzed by NADPH-dependent cytochrome P450 (CYP). *d*-Limonene (monocyclic hydrocarbon), and *beta*-myrcene (acyclic hydrocarbon) have been shown to be substrates (upon repeated administration) and competitive inhibitors of the same isoenzyme, specifically CYP2B1 and CYP2C11 [Miyazawa *et al.,* 2002] providing evidence that the inclusion of the acyclic hydrocarbon *beta*-myrcene in this group is appropriate. In a study of the induction of liver monooxygenase by *beta*-myrcene, liver microsomes from female rats treated *via* gavage with 1000 mg/kg bw/d *beta*-myrcene for one or three days were isolated. Activities of several markers of different cytochrome P450 enzymes were monitored including pentoxyresorufin-O-depentylase (PROD) and benzyloxy-resorufin-O-dealkylation (BROD), which are selective markers of CYP2B1. *beta*-Myrcene produced marked increases in the activities of both PROD and BROD. Levels of apoproteins CYP2B1/2B2 were increased 8.2 fold after repeated treatment with *beta*-myrcene. [De-Oliveira *et al.*, 1997a]. Limonene has also been shown to induce the members of the CYP2B family in several studies [Maltzman *et al.*, 1991; Hiroi *et al.*, 1995].

Both *d*-limonene and *beta*-myrcene, when incubated with liver microsomes from phenobarbital treated rats at concentrations of 0.05-2 *micro*M for *d*-limonene and 0.02-1 microM for *beta*-

myrcene, produced a concentration-dependent reversible inhibition of PROD [De-Oliveira *et al.*, 1997b].

Humans are continually exposed to limonene, myrcene and the other members throughout their lifetimes, *via* consumption of a traditional diet or inhalation of air. Extensive studies on *d*-limonene show rapid metabolism to polar oxidized metabolites, followed by conjugation and rapid excretion. *beta*-Myrcene has been shown to undergo similar pathways of metabolism and to induce the same cytochrome P450 enzymes (CYP2B1) as *d*-limonene, so it is appropriate that these monoterpene hydrocarbons and their structural analogs be evaluated in the same chemical category. The remaining substances in this category are expected to undergo similar pathways of metabolism given the close structural similarity.

Based on the pharmacokinetic, biochemical and metabolic data, it is concluded that members of this chemical category exhibit similar chemical and biochemical fate. The monoterpene substances in this group undergo detoxication *via* Phase I metabolism by CYP450 isoenzymes followed primarily by conjugation with glucuronic acid in Phase II metabolism and excretion in the urine. The physiochemical and toxicological properties of these substances are consistent with their known reactivity and common metabolic fate.

3　Test Plan

3.1　Chemical and Physical Properties

3.1.1　Melting Point

All the substances in this chemical category are liquids at ambient temperature. The melting point of d-limonene is reported to be -74.35°C [CRC Handbook of Chemistry and Physics, 1986; IPCS, 1998]. The reported melting point for dl-limonene is -97 $^{\circ}$C [CRC Handbook of Chemistry and Physics, 1986]. The calculated melting points for d-limonene, dl-limonene, terpinolene, myrcene, and dihydromyrcene are in the range from -29.5 to -66.1 $^{\circ}$C [MPBPVPWIN EPI Suite, 2000].

3.1.2　Boiling Point

Literature values from recognized sources are available for limonene (d or dl) (178°C @ 760 mmHg), terpinolene (186°C @ 760 mmHg), myrcene (167°C @ 760 mmHg), dihydromyrcene (165-168°C @ 760 mmHg) [CRC Handbook of Chemistry and Physics, 1986] and d-limonene (175.5-176°C @ 760 mmHg) and myrcene (10°C at 44 mmHg) [Merck Index, 1996]. Measured values reported for limonene (176°C @ 760 mmHg), terpinolene (185°C @ 760 mmHg), myrcene (172°C @ 760 mmHg) [FMA] and dihydromyrcene (168°C @ 760 mmHg) [CRC Handbook of Chemistry and Physics, 1986] are consistent with standard literature resource values. Additionally, the measured boiling point for sweet orange oil (176°C @ 760 mmHg) that contains greater than 90% limonene [Givaudan-Roure, 1991h] is in good agreement with the boiling point for limonene itself. There is excellent agreement between boiling points reported in the literature and measured values for each of the four substances in this chemical category.

Of the seven mixtures in this chemical category, sweet orange oil, terpenes and terpenoids, sweet orange oil, and terpenes and terpenoids, limonene fraction, being composed almost exclusively of limonene, should exhibit boiling points close to that for limonene itself. Of the

remaining mixtures that contain mainly $C_{10}H_{16}$ hydrocarbons; boiling points should be somewhat lower than those reflected by major constituents of the mixture. Similar conclusions can be reached for the boiling point of the volatile portion of terpenes and terpenoids, turpentine oil residue. The boiling point of the remaining mixture (terpenes and terpenoids, turpentine oil, limonene fraction, distillation residues, being a complex mixture of terpene hydrocarbons and oxygenated terpene hydrocarbons) is undefined.

3.1.3   Vapor Pressure

The measured vapor pressure values for d-limonene (1.43 mm Hg or 0.19 kPa at 20°C) [IPCS, 1998] is similar to the calculated vapor pressure (1.2 mm Hg or 0.16 kPa at 20 ^{o}C) reported elsewhere [FMA]. The calculated and measured vapor pressures for limonene are in good agreement with calculated values for isomeric and homologous monoterpene hydrocarbons (terpinolene, 0.5 mm Hg or 0.07 kPa at 20 ^{o}C; $beta$-myrcene, 1.5 mm Hg or 0.2 kPa at 20 ^{o}C; sweet orange peel oil, 0.9 mm Hg or 0.12 kPa at 20 ^{o}C) [FMA]. Model values [MPBPVPWIN EPI Suite, 2000] for d-limonene (1.59 mm Hg), dl-limonene (1.63 mm Hg), terpinolene (1.44 mm Hg), myrcene (2.4 mm Hg), dihydromyrcene (2.6 mm Hg) are closely correlated with other calculated and the measured values. Based on the above data the vapor pressures of the five monoterpene hydrocarbons fall in the range from 0.5 to 2.6 mm Hg. Vapor pressures for the remaining monoterpenoid hydrocarbon mixtures and the volatile portion of the monoterpene hydrocarbon mixture in this category are expected to fall within this range.

3.1.4   Octanol/Water Partition Coefficients

Measured log Kow values using OECD 117 guidelines are available for two substances. Both terpinolene and sweet orange oil exhibit log Kow values of 5.3 [Givaudan-Roure, 1996a; 1996b]. The calculated log Kow values as reported by Syracuse Research Corporation (SRC), for five chemical substances in this category are in the range from 4.83 to 4.88 [SRC], and are consistent with the experimentally measured values. The narrow range and the close agreements with the two measured values in this group indicate consistency and imply reliability. It is expected that the log Kow values for members of this category are in the range from 4.8-5.3.

3.1.5 Water Solubility

Measured values for limonene and terpenes and terpenoids, sweet orange oil are 13.8 mg/L at 25 °C [WSKOWWIN EPI Suite, 2000c] and 30 mg/L at 20 °C, respectively [Givaudan-Roure, 1991]. Experimental values for myrcene and terpinolene of 5.6 mg/L [WSKOWWIN EPI Suite, 2000a] and 9.5 mg/L [WSKOWWIN EPI Suite, 2000b] at 25 °C and 23 °C, respectively, are in good agreement with the value for limonene. Model calculated water solubility values for limonene (*d-* and *dl*) is 0.74 mg/L and the value for terpinolene, myrcene and dihydromyrcene is 1.87 mg/L, [WSKOWWIN EPI Suite, 2000d], indicating the conservative nature of the model predictions. The solubility of all members of this chemical category at 25 °C is expected to be in the range from 5 to 30 mg/L.

3.1.6 New testing required

No new testing required.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half-lives for the structurally defined terpenoid hydrocarbons in this chemical category, are in the range from 0.884 to 0.64 hours [AOPWIN EPI Suite, 2000]. These calculations are based on measured rate constants for radical reactions of OH, O_3 and NO_3 with monterpene hydrocarbons in this category [AOPWIN EPI Suite, 2000]. The short half-lives are predicted based on the abundant presence of reactive allylic hydrogens on members of this chemical category. Therefore these figures can be considered reliable. The photodegradation model was not applied to the seven mixtures. These mixtures are principally composed of the structurally defined hydrocarbons in this chemical category. As such, the photodegradation of the volatile portion of these mixtures is represented by the calculated photodegradation rates of its constituents.

3.2.2 Stability in Water

No hydrolysis is possible for any of the materials in this group. All are expected to be stable in aqueous solution.

3.2.3 Biodegradation

Three GLP experimental studies evaluating biodegradability are available for this group of substances using standard OECD protocols. Additional studies in soil horizons obtained from coniferous and deciduous forests [Misra *et al.*, 1996] provide a broader perspective on the biodegradation of monoterpene hydrocarbons in the environment.

Terpinolene was found to be biodegradable in two studies. In one study of inherent biodegradability, 80% biodegradation was reported using an OECD 302C guideline protocol after 28 and 31 days [Rudio, 1998]. In a study of ready biodegradability, 62.1% biodegradation was reported using an OECD 301B protocol after 28 days [Birch, 1996]). One study of the ready biodegradability of terpinolene by OECD 301F reported it to be not readily biodegradable (51% after 28 days) [Rudio, 1997] but the authors concluded that the high vapor pressure and low water solubility of terpene hydrocarbons leads to volatilization in the upper parts of the test vessel, thereby, limiting aerobic biodegradation.

Additional studies in extracts and slurries prepared from soils of coniferous and deciduous forest indicate rapid and complete biodegradation of limonene and terpinolene in a closed bottle test. Soil extracts from coniferous and hardwood watersheds were added to sealed flasks containing oxygen-saturated media that were preconditioned with limonene or terpinolene for 24 hours. Limonene and terpinolene underwent 100% biodegradation after approximately 1 day in acclimated medium and after day 8 in non-acclimated medium. The authors concluded the limonene and terpinolene are completely degradable in extracts prepared from watershed soils of coniferous or deciduous forests [Misra *et al.*, 1996].

In the soil regimen biodegradation tests, both limonene and terpinolene were found to be completely biodegradable within 8 days in unacclimated medium. Terpinolene was also found to

be readily biodegradable in two separate OECD studies. Given the close structural similarity of the members of this chemical category, it is reasonable to predict ready biodegradation for chemical substances and 6 of the 7 mixtures in this chemical category. In the remaining mixture, the monoterpene hydrocarbon fraction in the mixture containing appreciable quantities of polymeric terpenes is also expected to be biodegradable.

3.2.4 Fugacity

Transport and distribution in the environmental were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Trent University, 1999]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. Where measured values were available, these were used, but where they were not, calculated data from the EPIWIN series of programs were used. Given the similarity of the physical properties of the substances in this group, it is not unexpected that they would be predicted to exhibit similar distribution in the environment. The value of these calculations must be evaluated in the context that the substances in this chemical category are products of plant biosynthesis and are, therefore, ubiquitous in the environment. The model does not account for the influence of biogenic production on partitioning in the environment nor does it take into account biodegradation. Therefore, the relevance of fugacity calculations for these substances is questionable.

3.2.5 New testing required

No new testing required.

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

The 96 hour experimental LC50 values for limonene and terpinolene using fathead minnows in a flow-through system were reported to be 0.702 and 0.720 mg/L, respectively [Broderius, 1990]. Calculated 96 hour ECOSAR values are 0.221 mg/L (model input values of log Kow = 5.3, mp = -74.3 $^{\circ}$C, water solubility = 13.8 mg/L) for limonene (*d* and *dl*), 0.198 mg/L (model

input values of log Kow = 54.88, mp = -29 $^{\circ}$C, water solubility = 9.5 mg/L) for terpinolene, 0.198 mg/L (model input values of log Kow = 4.88, mp = -64 $^{\circ}$C, water solubility = 5.6 mg/L) for myrcene, and 0.201 mg/L (model input values of log Kow = 4.88, mp = -64 $^{\circ}$C, water solubility = 5.6 mg/L) for dihydromyrcene [ECOSAR EPI Suite, 2000].

Good correlation exists between the experimental and calculated 96 hour LC50 values, although model 96 hour LC50 values are always lower than measured values for the chemically defined substances in this category. Since limonene, terpinolene and *beta*-myrcene are the principal constituents of the mixtures in this chemical category, it is reasonable that they should have acute fish toxicities in the range of 0.2 to 1.0 mg/L.

3.3.2   Acute Toxicity to Aquatic Invertebrates

Seven experimental values are available for three members of this chemical category. The 48 hour EC50 for limonene with *Daphnia pulex* is reported to be 69.6 mg/L [Passino and Smith, 1987]. However, since the solubility of limonene is 13.8 mg/L at 25 $^{\circ}$C, the actual test concentration is not known. The 96 hour LC50 value for limonene with *Daphnia magna* was reported to be 0.577 mg/L (95% C.I. 0.496-0.672 mg/L) while the 96 hr EC50 was reported to be 0.421 mg/L. When these tests were repeated using limonene from a different supplier, the 96 hour LC50 was reported to be 0.924 mg/L [US EPA]. The 48 hour LC50 for myrcene with *Daphnia magna* was reported to be 31 mg/L and is also considered unreliable given limits of solubility [Waggy and Blessing, 1986]. For terpinolene, the 96 hour LC50 was reported to be 2.55 mg/L and the EC50 was reported to be 1.38 mg/L [US EPA]. In another acute toxicity experiment with limonene and myrcene using brine shrimp, the 48 hour LC50 values were determined to be 104.1 and 39.2 ppm (approximately 104.1 and 39.2 mg/L), respectively [Saleh *et al.*, 1998]. Calculated 48 hour LC50 values are 0.496, 0.612, 1.147 and 0.263 mg/L for limonene, terpinolene, myrcene and dihydromyrcene, respectively [ECOSAR EPI Suite, 2000].

The experimental and calculated acute invertebrate toxicity values for these substances indicate that all of the five chemical substances and the seven mixtures that are primarily composed of

these substances should have acute aquatic invertebrate toxicities on the order of 0.5 - 3.0 mg/L.

3.3.3   Acute Toxicity to Aquatic Plants

Experimental data for limonene and terpinolene are available for this chemical category. Both limonene and terpinolene were subjected to static 96 hour toxicity tests using green algae at test concentrations of 1.81 and 3.38 mg/L, respectively. Neither limonene nor terpinolene showed any significant inhibition at those test concentrations [Broderius, 1990]. Calculated 96 hour EC50 values for aquatic plants (*i.e.*, green algae) are lower than experimental values and are 0.360, 0.441, 0.813, and 0.194 mg/L for limonene, terpinolene, myrcene, and dihydromyrcene, respectively. The model should be considered extremely conservative given that experimental values are an order magnitude greater than calculated values. Each of the members of this chemical category are expected to all have acute aquatic plant toxicity exceeding 1.81 mg/L.

3.3.4   New Testing Required
No new testing required.

3.4 Human Health Data

3.4.1 Acute Toxicity

Oral and dermal LD50 values for five members (limonene, myrcene, terpinolene, dihydromyrcene and sweet orange peel oil) of this chemical category indicate a low order of both oral and dermal toxicity. All rabbit and rat dermal, and mouse and rat oral LD50 values exceed 4,000 mg/kg with the majority of values greater than 5,000 mg/kg [Levenstein, 1975; Moreno, 1972a, 1972b, 1972c, 1972d, 1973a, 1973b, 1980b; Tsuji *et al*., 1975a; Paumgartten *et al*., 1990]. Based on these data, it is concluded that all of the members of this chemical category are of very low acute toxicity.

3.4.2 *In vitro* Genotoxicity

Mutagenicity/genotoxicity testing has been performed on three members of this chemical category, including a complete battery of *in vitro* genotoxicity tests using limonene. No evidence of mutagenicity was observed when limonene [Heck *et al.*, 1989; Florin *et al.*, 1980; Muller, 1993; Haworth *et al*., 1983] was incubated with *Salmonella typhimurium* (SAL) strains TA98, TA100, TA102, TA1535, TA1537, and TA1538 with and without S-9 metabolic activation at concentrations up to and including 150,000 microgm/plate. Limonene did not induce chromosomal aberrations when incubated with Chinese hamster ovary cells at a concentration of 50-500 microgm/ml [Anderson *et al.*, 1990], nor did it induce sister chromatid exchanges in Chinese hamster ovary cells at concentrations of 16.2-162 microgm/ml [Anderson *et al.*, 1990]. In a mouse lymphoma forward mutation assay, limonene was negative in L5178Y cells with and without S-9 metabolic activation up to a maximum concentration of 100 microgm/ml [Heck *et al.*, 1989; Myhr *et al.*, 1990]. When incubated with Syrian hamster embryo cells up to 100 microgm/ml or 3 mM, limonene did not induce statistically significant cell transformation [Pienta, 1980; Rivedal *et al.*, 2000]. The effects of limonene on gap junction intercellular communications were also tested at concentrations up to 1 mM in Syrian hamster embryo cells, and showed no effects [Rivedal *et al.*, 2000].

In an *in vitro* chromosome aberration test with human lymphocytes, myrcene did not induce chromosomal aberrations at concentrations up to 1000 microgm/ml with and without metabolic activation [Kauderer *et al*, 1991]. When incubated with Chinese hamster ovary cells in a V79-HPRT Gene Mutation Assay, myrcene was not mutagenic with or without metabolic activation [Kauderer *et al*, 1991]. In sister chromatid exchange (SCE) tests with human lymphocytes, myrcene did not induce sister chromatid exchanges at concentrations up to 1000 microgm/ml with or without metabolic activation [Kauderer *et al*, 1991]. Additionally, there was no evidence of genotoxicity when myrcene was incubated with V79 and hepatic tumor (HPT) Chinese hamster cells at concentrations up to 500 microgm/ml in SCE assays [Roscheisen *et al*, 1991]. In fact, myrcene reduced the SCE inducing effect of S-9 mix activated cyclophosphamide in human lymphocytes and Chinese hamster ovary (CHO) cells, and aflatoxin B1 in V79 and HTC Chinese hamster cells in a dose dependent manner [Kauderer *et al*, 1991; Roscheisen *et al.*, 1991].

No evidence of mutagenicity was observed when sweet orange peel oil was incubated with *Salmonella typhimurium* (SAL) strains TA98, TA100, TA1535, TA1537, and TA1538 with and without S-9 metabolic activation at concentrations up to 5000 microgm/plate [Heck *et al.*, 1989 and Crebelli *et al.*, 1990]. In a mouse lymphoma forward mutation assay, sweet orange peel oil was positive in L5178Y cells with and without S-9 metabolic activation up to a maximum concentration of 75 microgm/ml but only at highly toxic concentrations. The authors noted that that positive results in this assay may be associated with changes in physiologic culture conditions (pH and osmolality) [Heck *et al.*, 1989]. Negative results were obtained with sweet orange peel oil in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45 [Kuroda *et al.*, 1989].

In eighteen separate *in vitro* tests on the mutagenicity and genotoxicity of limonene, myrcene, and sweet orange peel oil; the majority was negative, with the exception of the mouse lymphoma assay using sweet orange peel oil. This result is questionable given the culture conditions present and the negative results of the mouse lymphoma assay using limonene since limonene is the majority (greater than 90%) constituent of sweet orange peel oil. It is reasonable

to conclude that given the structural similarity between the members of this chemical category, the substances in this category exhibit low genotoxic potential *in vitro*.

3.4.3   *In vivo* Genotoxicity

Two *in vivo* genotoxicity assays are available for two substances in this chemical category. In an *in vivo* mammalian spot test, no evidence of mutagenicity was reported when mouse embryos were treated *in utero* with 215 mg/g bw/d limonene on days 10 -11 of gestation [Fahrig, 1984].

In an *in vivo* cytogenetic bone marrow assay, *beta*-myrcene (100, 500 or 1,000 mg/kg) was orally administered *via* gavage to up to four male and female Wistar rats. Corn oil was used as the negative control while cyclophosphamide (30 mg/kg *via* intraperitoneal injection) was used as the positive control. A mitotic inhibitor (colchicine 5 mg/kg ip) was injected 1 hour before sacrifice. At 24 or 48 hours, animals were sacrificed and bone marrow cells harvested. Evaluations included the mitotic index and the frequency of chromosomal aberrations. A dose-related increase in the mitotic index in bone marrow cells was reported for rats administered the test substance. The authors commented that this might be an interaction between *beta*-myrcene, which is known to induce CYP-450 enzymes, and colchicine, which arrests cell division at metaphase. *beta*-Myrcene may have increased the bioavailability of colchicine leading to the increase in mitotic index observed in the experiment. No significant increases in chromosomal aberrations were reported in the treated animals at either 24 or 48 hours. The authors concluded that given the results, *beta*-myrcene was not clastogenic to the rat when orally administered at dose levels up to 1000 mg/kg bw [Zamith *et al*., 1993]. Based on the results of this *in vivo* genotoxicity assay and the numerous *in vitro* genotoxicity assays, it is unlikely that any of these materials would exhibit a significant genotoxic potential *in vivo*.

3.4.4   Repeat Dose Toxicity

Orange oil was administered intragastrically to female B6C3F1 mice daily for 5 days at dose levels of 0, 625, 1250 or 2500 mg/kg bw to determine effects on humoral and cell-mediated immune responses. A host resistance assay (Listeria monocytogenes bacterial challenge) was

used to assess cell-mediated immunity while the antibody plaque forming cell response to sheep erythrocytes was used to measure humoral immunity. Other parameters evaluated included body weights, lymphoid organ weights and spleen cellularity. Orange oil had no effects on cell-mediated or humoral immune response at any dose level tested [Gaworski *et al*., 1994].

Groups of five mice of each sex were administered 0, 413, 825, 1650, 3300, or 6600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16-day period. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. All but one animal receiving 3300 or 6600 mg/kg bw/d limonene died within three days of study initiation. No treatment-related clinical signs were observed in mice receiving doses of 1650 mg/kg bw/d or lower [NTP, 1990].

Groups of five rats of each sex were administered 0, 413, 825, 1650, 3300, or 6600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16-day period. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. All but two females receiving 3300 or 6600 mg/kg bw/d limonene died within two days of study initiation. No treatment related clinical signs were observed in rats receiving doses of 1650 mg/kg bw/d or lower [NTP, 1990].

Groups of five young adult male F344/N rats each were administered *d*-limonene at dose levels of 0, 75, 150 or 300 mg/kg bw/d five days a week for 27 days. Observations included daily body weight, weekly food intake, liver and kidney weights and light microscopy and histology of liver and kidneys. Rats were examined for hyaline droplet formation, granular cast formation and chronic nephrosis. Two-dimensional gel electrophoresis evaluation of protein profiles was conducted on samples of kidneys in the 150 mg/kg dose group killed on day 6. Dose related increases in liver and kidney weights were reported for all dose levels. Renal effects were noted including protein profile changes, hyaline droplet formation, and accumulation of *alpha*-2-

globulin was reported. Chronic nephrosis was present in all kidneys of treated animals killed on day 27 [Kanerva *et al.*, 1987].

Groups of ten rats of each sex were administered 0, 150, 300, 600, 1200 or 2400 mg/kg bw/d *d*-limonene in corn oil by gavage once per day, five days a week for 13 weeks. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. Histological examinations were performed on all vehicle control and high dose animals and all female rats in the 1200 mg/kg group. Tissues examined included adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur, sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Kidneys were examined for all male rats. Ninety percent of female rats (9/10) and fifty percent of male rats (5/10) receiving 2400 mg/kg bw/d limonene died within the first week of the study. The final mean body weights of male rats receiving the three highest doses (600, 1200 or 2400 mg/kg bw/d) were reported to be 6%, 12%, or 23% lower than that of the controls, respectively. Rough hair coats, lethargy, and excessive lacrimation were observed for all animals at the two highest dose levels. Nephropathy was reported for all groups of male rats but a dose related increase in severity of the lesion was reported for the dosed groups. The nephropathy was characterized by degeneration of epithelium in the convoluted tubules, granular casts with tubular lumens, primarily in the outer stripe of the outer medulla, and regeneration of the tubular epithelium. Hyaline droplets were observed in the epithelium of the proximal convoluted tubules in all groups of male rats including vehicle controls. Upon further review to determine if there were differences in these findings between control and treated animals, the blinded slides revealed no definite differences in the accumulation of hyaline droplets [NTP, 1990].

Groups of ten mice of each sex were administered 0, 125, 250, 500, 1000 or 2000 mg/kg bw/d *d*-limonene in corn oil by gavage once per day, five days a week for 13 weeks. Animals

were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. Histological examinations were performed on all vehicle control and high dose animals. Tissues examined included adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. One of 10 males and 2/10 females administered 2000 mg/kg bw/d limonene and 1/10 females administered 500 mg/kg bw/d limonene died before the end of the study. Several other animals also died as a result of gavage error. Mean body weights were 10% lower than control for male mice and 2% lower than control for female mice for the two highest dose levels. An alveolar cell adenoma was reported in the lung of one female at the highest dose level. Clinical signs of rough hair coats and decreased activity were reported for the two highest dose levels [NTP, 1990].

Groups of five-week-old male rats received 0, 2, 5, 10, 30 or 75 mg/kg bw/d *d*-limonene daily *via* oral gavage for 13 weeks (5 days a week). Rats from selected dose groups were necropsied throughout the study (days 8-29), with all remaining rats necropsied at the end of the study. Rats were observed daily for toxicity signs. Body weights were taken daily. Linear regression analyses indicated increased relative kidney and liver weights at the two highest dose levels. Histological examination revealed changes characterized by hyaline droplet formation, granular casts and multiple cortical changes, all of which was classified as chronic nephrosis. Exacerbation of hyaline droplet formation was reported at the earliest necropsy eight days after administration at the 10 mg/kg bw/d dose level [Webb *et al.*, 1989].

d-Limonene was orally administered to Sprague-Dawley rats daily for 30 days at the following dose levels 0, 277, 554, 1385, or 2,770 mg/kg bw in order to investigate the effect on the fine structure of the liver, kidney and blood cells. No morphological changes of renal corpuscles and

tubular cells were observed. Some alterations were detected in the glomerular epithelium from the kidneys of rats treated at the highest dose level [Kodama *et al.*, 1977b, 1977c].

Groups of fifty male and fifty female rats each were administered 0, 75 or 150 mg/kg bw/d or 0, 300 or 600 mg/kg bw/d *d*-limonene, respectively, in corn oil by gavage once per day, five days a week for 103 weeks. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study; all vehicle control; all low dose female rats and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose male rat groups included adrenal glands, kidney, liver, spleen, and testis. Mean body weights for male rats administered 150 mg/kg bw/d *d*-limonene were generally 4-7% lower than vehicle controls from week 2 to study termination. Mean body weights of high dose females were generally 4-7% lower than vehicle controls from week 28 to study termination. No treatment related clinical signs were reported for the duration of the study. Survival of the high dose male group was significantly greater than that of the vehicle alone after week 81. Survival of the high dose female group was significantly lower than that of the vehicle controls after week 39. In the kidneys of male rats, dose-related increases were observed in the incidence of mineralization and epithelial hyperplasia. A dose-related increase in the severity of spontaneous nephropathy was reported in male rats administered limonene. Increased incidences in tubular cell hyperplasia and neoplasia were also reported in dosed male rats. Tubular cell adenoma incidence in high dose male rats and tubular cell adenoma or tubular cell

carcinomas (combined) in dosed male rats were significantly greater than vehicle controls. The authors determined that under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of *d*-limonene for male F344/N rats as shown by increased incidences in tubular cell hyperplasia, adenomas, and adeno-carcinomas of the kidney. There was no evidence of carcinogenic activity of *d*-limonene for female rats receiving 300 or 600 mg/kg bw/d [NTP, 1990].

Groups of fifty male and fifty female mice each were administered 0, 250, or 500 mg/kg bw/d or 0, 500 or 1000 mg/kg bw/d *d*-limonene, respectively, in corn oil by gavage once per day, five days a week for 103 weeks. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study, all vehicle controls, and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose groups include liver for female mice. Mean body weights for female mice administered 1000 mg/kg bw/d *d*-limonene were generally 5-15% lower than vehicle controls from week 28 to study termination. No treatment related clinical signs were reported for the duration of the study. Survival of the low dose male group was significantly lower than that of the vehicle controls by study termination. The authors determined that under the conditions of these 2-year gavage studies there was no evidence of carcinogenic activity of *d*-limonene for male or female B6C3F1 mice at the dose levels tested [NTP, 1990].

Evaluation of results of the NTP bioassay of d-limonene in male F344/N rats

It has been demonstrated that renal lesions, which were observed in the NTP study, resulted from the accumulation of aggregates of *alpha-*2 microglobulin (a low molecular-weight protein synthesized in the liver) and limonene-1,2-epoxide in the P2 segment of the renal proximal tubule. These aggregates prevent lysosomal degradation, which leads to accumulation in the cytoplasm of the protein or the protein-chemical complex, which leads to single cell necrosis, and ultimately, renal neoplasia (Lehmann-McKeeman *et al.*, 1990; Hildebrand *et al.*, 1997). This phenomenon has only been observed in the male F344/N rat (Strasser, 1988; Borghoff *et al.*, 1990).

The gene that encodes *alpha-*2 microglobulin has been isolated and the sequence deduced (Untermann *et al.*, 1981). These proteins are expressed in the liver under hormonal control (Roy and Neuhaus, 1967; Wang and Hodgetts, 1998). *alpha-*2 microglobulin belongs to the *alpha-*2 microglobulin super family of proteins that are characterized by a unique hydrophobic binding pocket. The lesions do not develop in the female F344/N rat or in humans (Bucher *et al.*, 1986). Subsequent investigations have shown that the *alpha-*2 microglobulin nephropathy found in the F344/N male rat only develops in mammals that express the hepatic form of *alpha-*2 microglobulin (Swenberg, 1989) unlike other strains of rats (Dietrich and Swenberg, 1991), mice (Bucher *et al.*, 1986; Lehman-McKeeman, 1994) and dogs (Webb, 1990).

Transgenic mice that express rat *alpha-*2 microglobulin were tested for their ability to form hyaline droplets and develop nephropathies similar to their adult male rat counterparts (Lehman-McKeeman and Caudill, 1994). This study involved male F344 rats as positive control, transgenic C57BL/6J mice as experimental group and native C57BL/6 mice as negative controls. The animals at age 70-75 days were placed in metabolic cages and received 150 mg/kg bw per day *d*-limonene in corn oil by gavage for three days. Limonene was used to induce renal nephropathy in adult male rats, as it was shown to be a potent inducer in the NTP studies (EPA, 1991) [NTP, 1990]. Twenty-four (24) hours after the last dose, the animals were sacrificed and the kidneys analyzed for evidence of nephropathy. Hyaline droplet formation was evaluated on a subjective scale, size and intensity (0-4) multiplied by tubular loading (0-3) for an overall scale of 0-12 with 12 being the most severe. In the absence of *d*-limonene, the control

groups transgenic mice and rats showed a hyaline droplet score of 1+/- 0 and 6 +/- 0.5, respectively. The test transgenic mice and rats showed a hyaline droplet score of 2.5 +/- 0.3 and 11 +/- 1.3, respectively upon dosing with *d*-limonene. The native mice developed no signs of hyaline droplet formation and tested negative for presence of *alpha*-2 microglobulin in their urine. The authors assert that based on the data presented "*alpha*-2 microglobulin is the only protein that is involved in the etiology of hyaline droplet nephropathy".

An increase in the kidney-type-*alpha*-2 microglobulin was seen in the urine of male Sprague-Dawley rats when these animals were administered greater than 30 mg/kg/day of *d*-limonene for 7 days by gavage. The increases in the urinary kidney-type-*alpha*-2 microglobulin are dose-dependent and parallel-elevated accumulation in the kidney cells (Saito, 1996).

While humans produce low molecular weight serum proteins, which are reabsorbed by the kidney, there is no evidence that *alpha*-2 microglobulin is produced (Olson, 1990). Urine collected from adult male F344 rats and humans revealed no evidence indicative of *alpha*-2 microglobulin production in humans (Olson, 1990).

It is unknown whether any human serum proteins possess a binding site similar to that of *alpha*-2 microglobulin. Although this is a possibility, it appears remote, since female rats and mice do not show the renal changes noted in male rats exposed to limonene. It should be noted that there is a class of human proteins referred to as the *alpha*-2 microglobulin related proteins. They appear to have no functional relationship to the adult male rat urine proteins. The human protein has a higher molecular weight, 25 kDa and is a component of a neutrophil gelatinase complex (Kjeldsen *et al.*, 2000; Triebel *et al.*, 1992). An extensive review of the current scientific literature and genome databases reveals no native protein or biological entity that acts as a nephropathy agent like mature male rat *alpha*-2 microglobulin. The accumulated evidence indicates that it is the unique anatomical, physiological, and biochemical properties of the male rat kidney, especially the proximal convoluted tubule, that allows *d*-limonene to interfere with renal processing of the strain-specific *alpha*-2 microglobulin. Therefore, this process is not predictive of human carcinogenicity. In a comprehensive review of *alpha*-2 microglobulin

nephropathy and associated renal tubule tumors produced in the male F344/N rat exposed to limonene and other simple chemical substances (e.g. isophorone, decalin and methyl isobutyl ketone), it was concluded that the F344/N rat is not an appropriate model for assessing human renal carcinogenic risk (EPA, 1991). After careful review, it has been concluded that the mechanisms leading to the renal carcinogenic findings in the F344/N male rat are largely known and strongly indicate that the nephropathy associated with *d*-limonene have no significance for human risk assessment (Burdock *et al.*, 1990).

Groups of ten Sprague Dawley rats of each sex were administered 0, 240, 600 or 1500 mg/kg bw/d sweet orange oil in 1% methyl cellulose by gavage daily for 30 days. Observations included survival, clinical observations, body weights, food consumption, clinical pathology, gross pathology, organ weights and histopathology. No treatment related effects were reported for survival, clinical observations, body weights or food consumption. Decreases in glucose levels related to treatment were reported in the mid-dose females and high-dose males and females. Increases in serum albumin and total serum protein were observed in all treated females and the high-dose males. Histopathology revealed treatment related lesions in the nonglandular stomach of the high-dose males and females and in the kidney of all treated male groups. Kidney weights were also increased in all of the treated male groups and in the high-dose female group. Liver weight increases related to treatment were reported for the high-dose females and all treated male groups. The authors concluded that the no-observed-effect-level (NOEL) under conditions of this study was less than 240 mg/kg bw/d for both male and female rats. The authors noted that the kidney changes observed in the male rat at all dose levels were expected given the known interaction between limonene and *alpha*-2-microglobulin (Serota, 1990). Limonene is the principal constituent of orange oil [Bauer and Garbe, 1985].

Several repeat dose studies have been conducted and demonstrate that this category of monoterpenoid hydrocarbons is of low toxic potential. The kidney changes seen in male rats administered limonene have been well characterized, and are known to be specific to the male rat and of no significance in human risk assessment.

3.4.5 Reproductive Toxicity

beta-Myrcene was administered *via* gavage to female Wistar rats at dose levels of 250, 500, 1000 and 1500 mg/kg bw/d of *beta*-myrcene from the 15th day of gestation until weaning of the offspring which was day 21 postnatal. Reproductive capacity was assessed in the exposed offspring upon reaching maturity (120 days). Mortality, weight gain and post-natal development were evaluated (see section 3.4.6 Developmental/Teratogencity Toxicity below for developmental effects) Fertility was impaired in female offspring exposed to 1000 or 1500 mg/kg bw/d of *beta*-myrcene [Delgado *et al*., 1993b].

Three experimental groups (15 male and 45 female Wistar rats per group) were administered *beta*-myrcene dissolved in peanut oil *via* gavage at dose levels of 0, 100, 300, or 500 mg/kg bw/d. The exposure period was 91 days prior to and during the mating period for the males and 21 days prior to and during the mating period for females, pregnancy, and lactation until 21 days post parturition. All parent animals were evaluated for weight development, mortality, and toxicity signs. Pregnant females were also evaluated for weight gain, spontaneous abortions, dystocia and prolonged duration of pregnancy. All males were sacrificed and decapitated at the conclusion of mating. One third of the females in each dose group were sacrificed at day 21 of pregnancy. All fetuses were examined for skeletal abnormalities. After the remaining pregnant females gave birth, the offspring were weighed and examined for development, specifically, incisor eruption, fur, downy hair, and eye opening. At weaning on day 21, all mothers were sacrificed and necropsied.

Neither deaths nor signs of toxicity were reported in male rats at any dose level. No statistically significant differences in body weight gain were reported between control and test animals. A slight increase in liver and kidney weights was reported for treated male (highest dose only) and female rats. No morphological alterations of the liver or testis tissue were revealed upon microscopic examination. No effects were reported on the number of spermatids in the testis or on the number of spermatozoa in the cauda epididymis. No adverse effects on body weight gain and no other signs of toxicity were observed in treated female rats during the pre-mating or

mating periods. No treatment related effects were reported on fertility as measured by the mating index and pregnancy index upon comparison to controls. At the highest dose level, a slight increase in the resorption rate and a parallel decrease in the ratio of live fetuses per implantation site were reported.

Increases in the occurrence of fetal skeleton abnormalities between control and treated groups were reported at the 500 mg/kg bw/d level. No adverse effects were reported on duration of pregnancy, labor, pup mortality, and maternal or offspring weight changes. Slight delays in incisor eruption (300 mg/kg bw/d) and eye opening (100, 300 mg/kg bw/d) were reported but were not dose-related. The authors attributed the increase in skeletal abnormalities at the highest dose level tested to known strain-specific anomalies including increases of dislocated sternums, and lumbar extra ribs. The authors concluded that the NOAEL for toxic effects on fertility and general reproductive performance *via* the oral route was 300 mg /kg bw/d for *beta*-myrcene [Paumgartten *et al.*, 1998].

Groups of ten female rats were orally administered sweet orange oil *via* gavage at dose levels of 0, 375, 750 or 1500 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weight, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on Day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 postpartum, any pups dying during the lactation period were necropsied. No deaths occurred at any dose level. Statistically significant numbers of rats from all three dose groups experienced excess salvation during the pre-mating and gestation periods, and during the lactation period for high-dose animals. The dosed rats had decreased weight gains compared to the control rats during the seven day premating period. Absolute and relative maternal food consumption was significantly decreased for the 750 and 1500 mg/kg bw/d dose groups during the seven day premating period. No treatment related effect on mating performance or fertility was reported at any dose level. A significant increase in stillbirths and pup deaths was reported for the highest dose group compared to the control group (See 3.4.6

Developmental/Teratogencity Toxicity below for developmental effects) [Hoberman *et al.*, 1989].

Given the results of three reproductive toxicity assays using sweet orange peel oil predominantly composed of *d*-limonene and *beta*-myrcene, it may be concluded that the substances within this chemical category exhibit low reproductive toxicity potential.

3.4.6   Developmental/Teratogenicity Toxicity

Four groups of twenty Wistar female rats each were administered 0, 591 or 2,869 mg/kg bw/d *d*-limonene on days 9-15 of gestation. At the highest dose level, increases in maternal mortality and decreases in maternal and fetal body weights were reported. Additionally at the highest dose level, delayed ossification of fetal metacarpal bones and proximals phalanx and decreased weights of the thymus, spleen, and ovaries were reported. The NOAEL for both maternal and offspring toxicity was reported to be 591 mg/kg bw/d [Tsuji *et al.*, 1975b].

Pregnant Japanese white rabbits were administered 0, 250, 500 or 1,000 mg/kg bw/d *d*-limonene on days 6 to 18 of gestation. Increased maternal mortality was reported at the highest dose level. Significant decreases in maternal body weight gain and food consumption were temporarily observed at the 500 and 1,000 mg/kg bw/d dose levels. No treatment related effects were reported for the offspring. The NOAEL for maternal toxicity was reported to be 250 mg/kg bw/d. The NOAEL for offspring toxicity was reported to be greater than 1,000 mg/kg bw/d [Kodama *et al.*, 1977a].

Pregnant ICR mice were administered 0, 591 or 2,363 mg/kg bw/d *d*-limonene on days 7 to 12 of gestation. Significant decreases in body weight gain were reported for pregnant ICR mice administered the highest dose level of *d*-limonene. In the offspring, increased incidence of fused ribs compared to that of the controls, delayed ossification of some bones and decreased body weight gain were reported at the highest dose level tested. The NOAEL for both maternal and offspring toxicity was reported to be 591 mg/kg bw/d [Kodama *et al.*, 1977a].

Pregnant Wistar rats were administered 0, 250, 500 or 1,200 mg/kg bw/d *beta*-myrcene on gestational days 6-15. The vehicle was corn oil. Decreased maternal weight gain was reported at the 1,200 mg/kg bw/d dose. Increased fetal skeletal malformations were reported at the 1,200 mg/kg bw/d dose level. The NOAEL for both maternal and offspring toxicity was reported to be 500 mg/kg bw/d [Delgado *et al.*, 1993a].

beta-Myrcene was administered *via* gavage to female Wistar rats at dose levels of 250, 500, 1,000 or 1,500 mg/kg bw/d from pregnancy day 15 until weaning of the offspring, which was day 21 postnatal. Mortality, weight gain and post-natal development were evaluated. Reproductive capacity was assessed in the exposed offspring upon reaching maturity (120 days). No adverse effects were noted in the offspring at the lowest dose level tested. Decreased body weight, increased perinatal mortality, and delayed developmental landmarks were noted at the 500, 1000 and 1500 mg/kg bw/d dose levels. Fertility was impaired in female offspring exposed to the two highest doses of *beta*-myrcene. The NOAEL for peri- and post-natal development was set at 250 mg/kg bw/d [Delgado *et al.*, 1993b].

Groups of ten female rats were orally administered sweet orange oil *via* gavage at dose levels of 0, 375, 750 or 1,500 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weight, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 postpartum, any pups dying during the lactation period were necropsied. No deaths occurred at any dose level. Statistically significant numbers of rats from all three dose groups experienced excess salvation during the premating and gestation periods, and during the lactation period for high-dose animals. The dosed rats had decreased weight gains compared to the control rats during the seven-day premating period. Absolute and relative maternal food consumption was significantly decreased for the 750 and 1500 mg/kg bw/d dose groups during the seven day premating period. No treatment related effects were reported on maternal body weight, changes in body weight, and absolute and relative feed consumption during the lactation

period. No treatment related effect on mating performance or fertility was reported at any dose level. A significant increase in stillbirths and pup deaths was reported for the highest dose group compared to the control group. The treatment with sweet orange oil had no effect on the incidence of malformations or gross lesions in the pups. The NOAEL for administration of sweet orange peel oil under the conditions of this study was reported to be less than 375 mg/kg bw/d for maternal toxicity and 750 mg/kg bw/d for offspring development [Hoberman *et al.*, 1989].

Given the results of six developmental toxicity assays using limonene, sweet orange oil and *beta*-myrcene, it may be concluded that the substances within this chemical category exhibit low developmental toxicity potential.

3.4.7   New Testing Required

No new testing required.

3.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 5989-27-5(*d-*) **CAS No. 138-86-3** (*dl-*) Limonene	A	A	A, Calc	Calc	A
CAS No. 586-62-9 Terpinolene	Calc	A	Calc	A, Calc	A
CAS No. 123-35-3 Myrcene	Calc	A	Calc	Calc	A
CAS No. 2436-90-0 Dihydromyrcene	Calc	A	Calc	Calc	Calc
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	NA	R	NA	NA	NA
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	NA	A	NA	NA	NA
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	NA	A	Calc	A	A
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	NA	R	NA	NA	NA
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	NA	R	NA	NA	NA
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	NA	R	NA	NA	NA
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	NA	R	NA	NA	NA

Chemical	Environmental Fate and Pathways			
	Photodegra-dation	Stability in Water	Biodegra-dation	Fugacity
CAS No. 5989-27-5(*d-*) **CAS No. 138-86-3** (*dl-*) Limonene	**Calc**	**NA**	**A**	**Calc**
CAS No. 586-62-9 Terpinolene	**Calc**	**NA**	**A**	**Calc**
CAS No. 123-35-3 Myrcene	**Calc**	**NA**	**R**	**Calc**
CAS No. 2436-90-0 Dihydromyrcene	**Calc**	**NA**	**R**	**Calc**
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	**R**	**NA**	**R**	**R**
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	**R**	**NA**	**R**	**R**
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	**R**	**NA**	**R**	**R**
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	**R**	**NA**	**R**	**R**
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	**R**	**NA**	**R**	**R**
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	**R**	**NA**	**R**	**R**
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	**R**	**NA**	**R**	**R**

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 5989-27-5(*d-*) **CAS No. 138-86-3** (*dl-*) Limonene	**A, Calc**	**A, Calc**	**A, Calc**
CAS No. 586-62-9 Terpinolene	**A, Calc**	**A, Calc**	**A, Calc**
CAS No. 123-35-3 Myrcene	**Calc**	**A, Calc**	**Calc**
CAS No. 2436-90-0 Dihydromyrcene	**Calc**	**Calc**	**Calc**
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	**R**	**R**	**R**
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	**R**	**R**	**R**
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	**R**	**R**	**R**
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	**R**	**R**	**R**
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	**R**	**R**	**R**
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	**R**	**R**	**R**
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	**R**	**R**	**R**

Chemical	Human Health Data					
	Acute Toxicity	**Genetic Toxicity** *In Vitro*	**Genetic Toxicity** *In Vivo*	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
CAS No. 5989-27-5(*d-*) **CAS No. 138-86-3** (*dl-*) Limonene	A	A	A	A	R	A
CAS No. 586-62-9 Terpinolene	A	R	R	R	R	R
CAS No. 123-35-3 Myrcene	A	A	A	R	A	A
CAS No. 2436-90-0 Dihydromyrcene	A	R	R	R	R	R
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	R	R	R	R	R	R
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	A	A	R	A	A	A
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	R	R	R	R	R	R
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	R	R	R	R	R	R
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	R	R	R	R	R	R
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue.	R	R	R	R	R	R
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	R	R	R	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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